Ecotoxicology and Environmental Safety 163 (2018) 37–46

Contents lists available at ScienceDirect

Ecotoxicology and Environmental Safety

journal homepage: www.elsevier.com/locate/ecoenv

Physiological and behavioural responses to acid and osmotic stress and effects of *Mucuna* extract in Guppies

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ARTICLE INFO

Keywords:
Stress response
Salinity
pH
Plant extract
Cortisol
Biomarkers

ABSTRACT

Variation in pH (acidification) and salinity conditions have severe impact at different levels of biological organization in fish. Present study focused to assess the effects of acidification and salinity changes on physiological stress responses at three different levels of function: i) hormonal and oxidative response, ii) osmoregulation and iii) reproduction, in order to identify relevant biomarkers. Second objective of the study was to evaluate the efficacy of plant (*Mucuna pruriens*) extract for alleviating pH and salinity related stress. Guppies (*Poecilia reticulata)* were exposed to different pH (6.0, 5.5, 5.0) and salinity (1.5, 3.0, 4.5 ppt) for 7, 14 and 21 days. Following exposure to stress for respective duration, fish were fed diet containing methanol extract of *Mucuna* seeds (dose 0.80 gm/kg feed) for 7, 14 and 21 days to measure their possible recovery response. Stress hormone (cortisol), hepatic oxidative stress parameters [superoxide dismutase (SOD), catalase (CAT), glutathione reductase (GRd), glutathione peroxidise (GPx), glutathione S-transferase (GST), malondialdehyde (MDA), glutathione (GSH)], gill osmoregulatory response (Na^+-K^+ATPase activity), sex steroid profiles and mating behaviours (gonopodial thrust and gestation period) were estimated. Cortisol and MDA levels increased with dose and duration of acid and salinity stress, and cortisol levels were higher in males than in females. Effect on Na^+-K^+ATPase activity was more intense by salinity stress rather than pH induced stress. Both acid and salinity stress reduced sex steroid levels, and mating response was highly affected by both stresses in a dose- and duration-dependent manner. *Mucuna* treatment reduced stress-induced alteration of cortisol, MDA, Na^+-K^+ATPase activity and reproductive parameters. Dietary administration of *Mucuna* seed extract decreased the intensity of environmental stressors at all three functional levels. *Mucuna* treatment was more effective against salinity stress than acid stress. Thus, cortisol, oxidative stress marker MDA and Na^+-K^+ATPase could be effective indicators for acid and salinity stress in wild and domestic fish populations. Dietary administration of *Mucuna* extract may limit the detrimental effects of acidification and salinity variations that are the inevitable outcomes expected under global climate change conditions.

1. Introduction

Pollution and temperature changes are increasingly exposing aquatic systems to water acidification and salinity alterations throughout the world (Guinotte and Fabry, 2008; Feely et al., 2010; Vaz et al., 2015). Such environmental stressors are affecting wild and farmed fish raised in semi-natural conditions, which may result in severe loss of biodiversity and cause economic losses in the near future (Williams and Rota, 2010). However, the physiological and reproductive responses of freshwater fish to such stressors are still poorly understood.

Most fish species survive in a narrow range of pH and salinity. Any deviation from the optimal range of pH or salinity or both can disrupt their physiological functions (Whitney et al., 2016; Mubarik et al., 2015; Serrano et al., 2010), eventually, alter their life history traits such as growth (Ong et al., 2015) and reproduction (Kwong et al., 2014). Recent studies helped deciphering information on the hyper-hypo-osmoregulators (Kelly et al., 2007), and the physiological stress responses

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https://doi.org/10.1016/j.ecoenv.2018.07.053
Received 22 March 2018; Received in revised form 11 July 2018; Accepted 13 July 2018

to pH and salinity changes (Katoh et al., 2008; Scott et al., 2008; Tipsmark and Madsen, 2009). Exposure to suboptimal pH and salinity levels leads to a rapid alteration in the cascade of molecular and physiological responses (Makrinos and Bowden, 2016; Xu et al., 2015). The primary responses involve hormonal parameters (cortisol secretion), secondary responses include oxidative stress responses (different biomarkers of oxidative stress and damages) and osmotic regulation responses (Na^+-K^+ATPase activity), followed by tertiary response such as reproductive changes (Xu et al., 2015).

During the primary response, cortisol mediates a range of physiological and behavioural responses against a variety of environmental stressors (Pottinger, 2017). Some of these changes can promote survival through increased metabolism and detoxification processes. The secondary response to suboptimal pH or salinity may be enhanced free radicals causing oxidative damages (Sies, 2016). Exposure to acute and/or chronic stressors can increase oxidative stress and result in irreparable damages to cell membranes, inactivation of several vital enzymes through the alteration of different transcription factor (Moniruzzaman et al., 2018). The effective control and rapid elimination of reactive oxygen species (ROS) is essential to the proper functioning, survival and reproduction of the aquatic organisms.

One of the major challenges to improve our understanding of the stress responses of fish to these stressors is to identify simple and reliable biomarkers of oxidative stress in fish (Lushchak, 2011; Mukherjee et al., 2017a, 2017b). Enzymes involved in antioxidant defences include radical scavenging enzymes such as catalase (CAT), superoxide dismutase (SOD) and glutathione peroxidase (GPX) (Klein et al., 2017; Halliwell and Gutteridge, 2006). Small water-soluble antioxidant compounds such as glutathione (GSH) is also one of the most reliable free radical scavenger of cells (Birben et al., 2012). Lipid peroxidation and malondialdehyde (MDA) levels are a direct reflection of the amount of oxidative damages as well. The activity of the membrane-bound enzyme Na^+-K^+ATPase changes accordingly with the amount of lipid peroxidation (Babu et al., 2006). This ultimately alters membrane function and permeability that may lead to destruction of cells or whole cell systems (Rind et al., 2017). CAT, SOD, GPX, GSH, MDA and Na^+-K^+ATPase activities are thus good potential biomarkers of saline and pH stressors but experimental studies comparing their relative intensity of response at different stress doses are still rare.

Environmental stressors can also affect fish reproduction (tertiary response). pH and salinity stress have been found to affect sperm and egg maturation, reproductive development, egg survival and fertilization in different aquatic species (Miller et al., 2015; Dadras et al., 2017; Allen et al., 2017). Alterations in different reproductive attribute may thus be important biomarkers for such environmental stress in fish. In this study, we performed a comprehensive characterization of physiological responses at different functional levels and aimed at identifying significant biomarkers regarding the effects of pH and salinity using an experimental approach in controlled conditions.

Some antioxidant molecules are known to have positive effects in alleviating these stress responses. Plant extracts in the dietary supplements have gained interest recently due to their nutritional values and positive effects on growth and reproductive performance in many fish species (Chakraborty and Hancz, 2011; Chakraborty et al., 2014). Medicinal plant *Mucuna pruriens* contains high amounts of proteins and carbohydrates and is a rich source of macro- and microelements. Alcoholic extracts of *Mucuna* seeds were shown to have potential antioxidant activity to regulate stress-induced lipid peroxidation (Madhyastha et al., 2011). Different preparations of *Mucuna* were recently used for the management of several free radical-mediated diseases (Rai et al., 2017). However, the effects of *M. pruriens* on stress responses antioxidant enzymes and reproduction in fish in response to environmental pH and salinity stress are not yet documented anywhere.

To test the effects of saline and pH stressors on the stress response of fish at different biological levels and the effects of *Mucuna* in modulating such stress responses, we used the guppy, *Poecilia reticulata* as a model species. *P. reticulata* is a popular freshwater species with high ornamental value and is an important biological tool to study the reproductive physiology throughout the world (Kavitha and Subramanian, 2011), because of its viviparity and short reproductive period (Guevara-Fiore and Endler, 2018). Though they live in almost every freshwater body near the coastal fringes, guppies have low tolerance to brackish water and found to colonize low salinity brackish habitats. In the wild and in captive conditions, their optimal pH is 6.5–8.0 and optimal salinity levels are about 2 ppt but beyond 3 ppt the survival rate decreases significantly (Kavitha and Subramanian, 2011).

The first objective of the study was to test the physiological consequences of pH and salinity stressors on guppy responses at three functional levels. First, we recorded the alterations in cortisol secretion (hormonal response). Second, we recorded the status of oxidative stress in the hepatic tissue (oxidative response), as well as the Na^+-K^+ATPase activity in gill (osmoregulatory response). Third, we recorded the reproductive response by measuring sex steroid profiles and mating behaviour (gonopodial thrust in males and gestation period in females) to determine the impacts of altered pH and salinity and identify the central biomarkers of eco-physiological stress. The second objective of the study was to evaluate the efficacy of the *Mucuna* plant extracts for alleviating pH and salinity stress at these three biological levels in guppies to assess the efficacy of *Mucuna* treatment to maintain the biomarkers at equilibrium.

2. Materials and methods

2.1. Model species and acclimation

Guppies were purchased from the local market and kept into large sized tank (180 × 90 × 60 cm) for ten days for acclimatization. During the acclimatization period, fish were fed an artificial diet containing 30% crude proteins (Tetra Bits Complete, Tetra). Throughout the entire period of acclimatization and experiment, all fish were maintained under constant temperature (T = 27 ± 0.5 °C), similar photoperiod (14 L: 10 D), optimum hardness (8.5 ± 0.8) and dissolved O_2 (6.5 ± 0.5 mg/L). Salinity ranges (0.1 ± 0.05) were also checked for all the fish during the acclimatization period and for control fish throughout the experiment. Similarly, the pH ranged between 6.7 and 6.9 for all the fish during acclimatization period and throughout the experiment schedule for control fish. During the entire experimental duration, levels of ammonia (< 4 ppm), nitrate (< 30 ppm), and nitrite (< 1.0 ppm) were measured daily using aquarium test kits. If levels exceeded the aforementioned limits, a 50% water change was performed. Otherwise, 25% of the water was changed every other day. Detritus were removed from the experimental systems through siphoning. Proper care was taken to avoid any sudden changes in temperature, salinity and pH during acclimatization period. No mortality was observed during the entire course of the experiment.

2.2. Experimental design

After 10 days of acclimatization, fish were divided into two categories: experimental and control. Experimental fish were again subdivided into two equal groups. One experimental group was exposed to pH stress and the other one to salinity stress (Fig. 1). The control category was not exposed to any pH or salinity stress. The experiment was conducted in static water systems. Salinity and pH were measured daily and adjusted as required both in control and experimental groups. Such adjustments were conducted in stock water prior to addition to the experimental aquaria to minimize stress to the fish.

Tap water was first provided into a mixing tank in which the pH was regulated by the addition of 10% sulphuric acid using a peristaltic pump controlled by an automatic pH controller. The experimental aquaria (90 × 60 × 60 cm) were filled with this pH-adjusted water (± 0.05 the desired pH) from the mixing tank. Fish were exposed to